

Dermatologic and ocular diseases

Coprinus comatus (shaggy cap) is a potential source of aeroallergen that may provoke atopic dermatitis

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Background: Basidiospores are universal components in the air and established as important causes of respiratory allergies.

Recent reports indicate that aeroallergens may aggravate eczematous skin lesions in subjects with atopic dermatitis (AD).

Objective: The aim of the study was to investigate whether spores of *Coprinus comatus*, a species of basidiomycetes, may elicit delayed-type skin reactions in subjects with an atopic predilection, especially dermatitis.

Methods: Sixty-six study subjects were categorized in groups having AD or respiratory allergy with regard to the skin prick test (SPT) reactivity to *C comatus* extract. Twenty nonatopic individuals served as control subjects. Atopy patch tests (APTs) were performed with extract of *C comatus* spore-containing tissue at a concentration of 1.35 mg of protein per gram of petrolatum (Vaseline) and *C comatus* cap at a concentration of approximately 5 mg of protein per gram of petroleum jelly. APT reactions were evaluated after 48 and 72 hours.

Results: Of the subjects with AD completing the study, 12 (32%) of 38 showed a positive APT reaction, with 8 (57%) also having a positive SPT response to *C comatus*. Only 1 (9%) of 11 subjects with asthma had a positive SPT and APT response to *C comatus*. No positive test reaction was observed in the nonatopic control subjects or in subjects with respiratory allergy and negative SPT responses to *C comatus*.

Conclusion: Our results demonstrate that *C comatus* can induce delayed-type reactions in atopic individuals, particularly in those with AD. Because spores of *Coprinus* species are ubiquitous, basidiomycetes have to be considered as possible aeroallergens when investigating causes of eczematous skin lesions in AD. (*J Allergy Clin Immunol* 1999;104:836-41.)

Key words: Fungal spores, basidiomycetes, *Coprinus comatus*, aeroallergen, atopic dermatitis, atopy patch test, delayed-type reaction

Basidiomycetes (fleshy mushrooms) are the morphologically most advanced of all fungi. There are an estimated 20,000 to 25,000 species worldwide, including

Abbreviations used

AD: Atopic dermatitis

APT: Atopy patch test

CSCTE: *C comatus* spore-containing tissue extract

SPT: Skin prick test

puffballs, bracket fungi, toadstools, and jelly fungi.¹ Spores of basidiomycetes occur in the air in high concentrations in many parts of the world¹⁻⁴ and have also been recovered in remarkable quantities from house dust.^{5,6} Basidiospores were demonstrated to be important sensitizers and triggers of respiratory allergies.^{1,7-9}

In a recent study 23% of subjects sensitized by skin prick test (SPT) to 3 different basidiomycete extracts had clinical signs of atopic dermatitis (AD).⁹ Because this rate of basidiomycete-reactive individuals was unanticipatedly high, an association between basidiomycete allergens and AD was assumed. Although the pathophysiology of AD is not completely understood, it has been observed that some patients with AD have local eczematous skin eruptions after allergen exposure or after application of atopy patch tests (APTs).¹⁰⁻¹⁴ In addition, marked improvement of AD was observed in these patients after reduction of exposure to the relevant allergens.¹² APTs are epicutaneous patch tests containing aeroallergens, such as grass pollen, house dust mites, or cat dander, which are known to elicit IgE-mediated hypersensitivity. Although the role of IgE in AD is still a matter of controversy, it has been demonstrated that epidermal Langerhans' cells from subjects with AD express IgE receptors and that these cells are able to present the allergen to T cells from the peripheral blood.¹⁵⁻¹⁹ Therefore we hypothesized that basidiospores also may have the potential to evoke eczematous skin lesions after skin contact.

In this study we investigated whether components of *Coprinus comatus* elicit APT delayed-type skin reactions in atopic subjects, particularly those with AD. In addition, we examined the correlation between sensitization to *C comatus* and the air-exposed pattern of eczematous lesions. Because collection of *C comatus* spores, the characteristic of which is the rapid autolytic process of the mature fruiting body, is very difficult, and it has been shown that all parts of the mushroom contain similar allergens,¹ we used caps and an extract from the spore-containing tissue.

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METHODS

Source material and preparation of test solutions

C. comatus mycelia were obtained from a German mushroom supplier (Pilzbrut Dieskau, Zwintschöna, Germany). Under recommended conditions, fruiting bodies were produced in collaboration with the Institute of Botany, University of Bern. After collection, gills were separated, peeled, and frozen at -80°C . For extraction, 1 g of spore-containing tissue was ground in liquid nitrogen and prepared in 40 mL of 125 mmol/L ammonium bicarbonate buffer (pH 8.1) for 1 hour with stirring at 4°C . The homogenate was centrifuged at 80,000g for 30 minutes at 4°C , and the supernatant was lyophilized. White Vaseline, consisting of 0.5% urea, was used as the vehicle for APTs. One test cream contained 1.35 mg of protein of *C. comatus* spore-containing tissue extract (CSCTE) per gram of Vaseline prepared by dissolving lyophilized material in 0.5 g of paraffinum liquidum in a mortar with a pestle. The second test cream contained 0.25 g of nitrogen-frozen and ground *C. comatus* cap in 1 g of basic cream corresponding to approximately 5 mg of protein per gram of Vaseline. All preparations were filled in vials and stored at 4°C until used. In preliminary studies with CSCTEs at different protein concentrations, the most reactive concentration was found at 1.35 mg of protein per gram of Vaseline. Commercially available and standardized house dust mite test substances (*Dermatophagoides pteronyssinus*/D farinae 200 IR) were obtained from Stallergenes SA (Antony Cedex, France).

Study population

After obtaining informed consent, 86 subjects (57 females and 29 males) with a mean age of 30.5 years (range, 14-66 years) were enrolled in the study. All subjects underwent SPTs with a panel of common inhalant allergens (house dust mites, grass, birch and mugwort pollen, cat and dog epithelia, cockroach, *Alternaria alternata*, and *Cladosporium herbarum* [Allergopharma, Reinbeck, Germany]) and with *C. comatus* extract (5 mg of protein per milliliter) with reference to the recommendations of the European Academy of Allergy and Clinical Immunology.²⁰ These subjects were divided prospectively into 5 groups according to clinic and SPT reaction to *C. comatus* extract (Table I). Subjects of group 1 and 2 had AD but differed in SPT reactivity to *C. comatus*. Subjects with respiratory allergies and no signs of AD were listed in groups 3 and 4 according to their *C. comatus* SPT reactivity. Twenty nonatopic subjects formed the control group (group 5). AD was diagnosed by using the criteria of Hanifin and Rajka.²¹ Most individuals with AD had some eczematous skin lesions outside the test area. Clinical severity of AD in groups 1 and 2, as documented by the SCORAD index,²² averaged 21 (range, 7-58). In addition, clinical distribution of eczema with reference to the air-exposed pattern was also recorded.¹⁴

APT

Test substances were applied for 48 hours in patch test chambers (16 mm; Leukotest; Beiersdorf AG, Hamburg, Germany) on clinically uninvolved skin on the back. Short-acting antihistamines were discontinued for at least 7 days and oral corticosteroids or long-acting antihistamines were discontinued for at least 6 weeks before testing. Topical corticosteroids or UV therapy had not been used in the last 4 weeks at test application sites. To obtain minimal skin irritation, APT substances were applied without tape-stripping or skin abrasion. Vaseline with 0.5% urea and pure Vaseline served as negative controls. Reactions were read after 48 and 72 hours. For grading positive APT reactions, criteria of conventional contact allergy patch testing were used.²³

Total serum IgE levels

Serum levels of total IgE were measured by the ImmunoCAP FEIA (Pharmacia Diagnostics, Uppsala, Sweden) system according to the manufacturer's instructions. Serum samples were taken from each study subject and stored at -20°C until used.

Histology and immunohistochemistry

Punch biopsy specimens were taken from 6 subjects with positive APT responses to *C. comatus* after 72 hours and processed for standard histology and immunohistochemistry. The following antibodies were used for the immunophenotyping of infiltrating cells: anti-CD4 (clone MT310; concentration, 2.3 $\mu\text{g/mL}$), anti-CD8 (clone DK25; concentration, 1.5 $\mu\text{g/mL}$), and anti-HLA-DR (clone CR3/43; concentration, 2.7 $\mu\text{g/mL}$), all of which were obtained from Dako (Glostrup, Denmark). Isotype-matched immunoglobulins were used as negative controls. Briefly, 5- μm sections from the snap-frozen tissue blocks were cut on a cryostat, air-dried, and fixed in acetone for 10 minutes. Slides were rehydrated in Tris-buffered saline with 5% normal rabbit serum for 20 minutes and incubated with the primary antibody diluted in Tris-buffered saline with 0.5% casein and 5% normal rabbit serum for 60 minutes at room temperature. After washing, sections were incubated with a biotinylated rabbit-anti-mouse IgG (dilution, 1:200; E0354; DAKO) and thereafter with streptavidin-biotin complex-peroxidase (K0377; DAKO). Finally, sections were developed in 3,3'-diaminobenzidine- H_2O_2 , counterstained with hematoxylin, and mounted.

Statistical analysis

Statistical analysis was performed by using a matched case-control test (computing McNemar's χ^2) for calculation of the concordance of positive APT and positive SPT reactions. The Fisher exact test was used for the correlation of positive APT responses and head-neck distribution. Probability (*P*) values of less than .05 were considered significant.

RESULTS

An example of a representative positive APT response elicited by CSCTE is shown in Fig 1. One male subject of group 1 and one female subject of group 2 were excluded from the study. One individual experienced a diffuse flare-up reaction over the entire back 48 hours after test application, and the second subject dropped out because of nonspecific reactions to APT substances, including the negative control.

Thirteen (20%) of 64 subjects with an atopic disorder (groups 1-4), and of these 12 (32%) of 38 with AD (groups 1 and 2), exhibited a definitive positive delayed-type reaction to the *C. comatus* APT response after 72 hours. Seven (54%) of the 13 reactors revealed a response to *C. comatus* cap, 2 (15%) reacted to CSCTE, and 4 (31%) reacted to both *C. comatus* allergen preparations (Fig 2). Eight (61%) of the APT reactors belong to group 1 with a positive SPT response to *C. comatus*, and 4 (31%) belong to group 2 with a negative SPT response to *C. comatus*. APT scores after 72 hours averaged 1.47 in group 1 and 1.4 in group 2. One (8%) subject with a positive SPT response to *C. comatus* with asthma had a positive APT response (score of 1.0) to CSCTE (group 3). Although this subject had no AD, he reached a score of 9 points by using the system of Diepgen et al,²⁴ which determines minor features of AD. No positive APT responses were recorded in groups 4 and 5.



FIG 1. Positive APT reaction induced by 1.35 mg of CSCTE per gram of Vaseline.

Twenty (31%) of the atopic individuals, 14 (37%) with AD and 6 (23%) with a respiratory allergy, had a positive APT response to house dust mite allergen. Of them, 12 (60%) had positive SPT responses to house dust mite allergen. Fig 3 demonstrates that 8 (45%) of 18 of the reacting subjects with AD (groups 1 and 2) had positive responses to both the *C comatus* and house dust mite allergen APTs. Five individuals with respiratory symptoms but without AD reacted to the house dust mite allergen APT, whereas 1 subject reacted to the *C comatus* APT only.

The rate of APT reactors was significantly increased ($P = .017$) in patients who had an air-exposed pattern of AD. Seven (64%) of 11 had a positive APT response to *C comatus* compared with only 4 (15%) of 27 without this pattern. In addition, the APT score of 1.86 in subjects with an air-exposed pattern was considerably higher than in those without this aspect (APT score, 1.20). A statistically significant association ($P = .0073$) was also found between APT and SPT responses to *C comatus*. On the contrary, no statistical significance was detected between positive APT responses to *C comatus* and the SCORAD rating of these patients nor between positive APT responses and total IgE levels.

A typical example of the histologic and immunohistochemical analysis from a positive APT response to *C comatus* cap is depicted in Fig 4, A through C. Histologic examination revealed focal epidermal spongiosis and superficial predominantly dermal mononuclear cell infil-

TABLE I. Characteristics of the 86 participants of the 5 study groups

Group	No.	Diagnosis	SPT response* to <i>C comatus</i> extract	Mean total IgE, kU/L (range)
1	15†	AD	Positive	1727 (104-4413)
2	25†	AD	Negative	506 (90-2930)
3	11	Respiratory allergy	Positive	860 (46-2199)
4	15	Respiratory allergy	Negative	186 (8-797)
5	20	Negative control subjects	Negative	40 (4-79)

*Positive skin prick test response defined as a wheal of 3 mm or greater in diameter.

†One subject of each group dropped out.

trate with some eosinophils (Fig 4, A). Immunohistochemical staining demonstrated a predominant T-cell infiltration consisting of approximately two thirds CD4⁺ (Fig 4, B) and one third CD8⁺ cells. The majority of the mononuclear cell infiltrate was intensively stained for HLA-DR (Fig 4, C).

DISCUSSION

In recent years, basidiomycete spores have been recognized as important aeroallergens in atopic subjects, particularly those with respiratory allergies, in many parts of the world.^{1,7-9} *Coprinus* species (inky cap) are ubiquitous in North America and in Europe, where about 250 species have been described.²⁵ *C comatus* (shaggy cap) is a common mushroom in our area that produces peaks of spores from June until October.²⁶ The allergenic potential of different species of *Coprinus* has been demonstrated by positive skin test responses and RAST results.^{1,7,9,27-29}

In this study we showed that *C comatus* applied by APT either as ground cap or as CSCTE elicited eczematous skin lesions in unaffected skin areas in 32% of subjects with AD. Histologic and immunohistochemical findings of these lesions were consistent with an acute cutaneous lesion of AD. The predominance of CD4⁺ cells may reflect a *C comatus*-specific T-cell response, as suggested in previous studies.^{19,30-32} Most of the APT-reactive subjects had a positive SPT response to *C comatus*, indicating that IgE formation may facilitate antigen capture.^{17,18,32} However, even without detectable IgE to the *C comatus* extract in SPTs, 4 (16%) of 25 individuals with AD revealed a clear-cut positive APT response. This finding suggests that T-cell sensitization alone may be sufficient to induce an APT response.

Reliability of our *C comatus* test preparations has been demonstrated because none of the nonatopic control subjects or subjects with respiratory allergy and negative SPT responses to *C comatus* exhibited a positive APT response. The majority of subjects with positive APT responses reacted to the allergen preparation from *C comatus* cap. Previously, it has been demonstrated that *C comatus* cap contains allergens in quantities almost equal

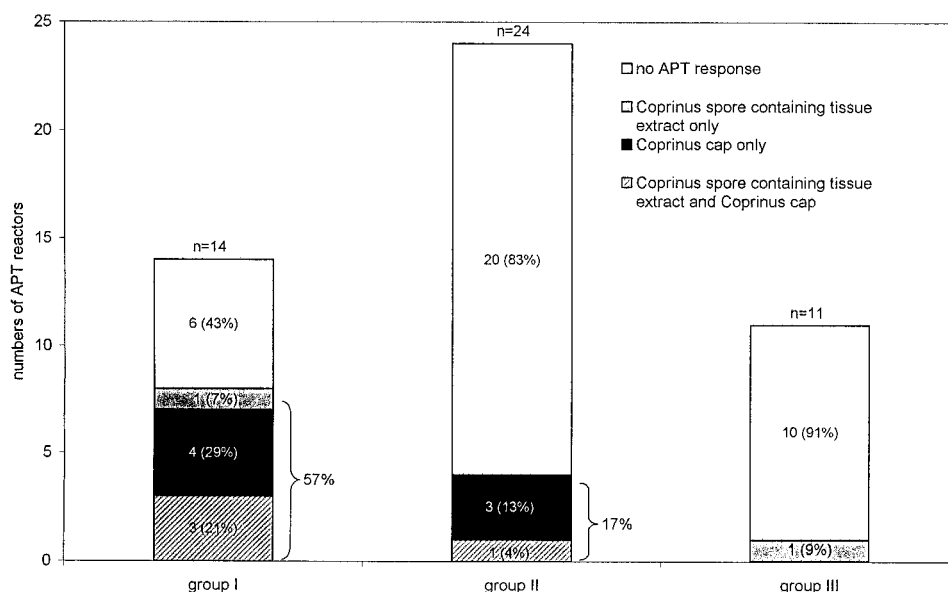


FIG 2. Number of APT-reactive subjects to *C. comatus* cap- and/or spore-containing tissue extract in groups 1 to 3. Eight (57%) of 14 of the subjects with positive SPT responses and 4 (17%) of 24 of the subjects with negative SPT responses had positive APT responses. None of the individuals with negative SPT responses and respiratory allergy (group 4) and none of the nonatopic control subjects (group 5) reacted to the APTs.

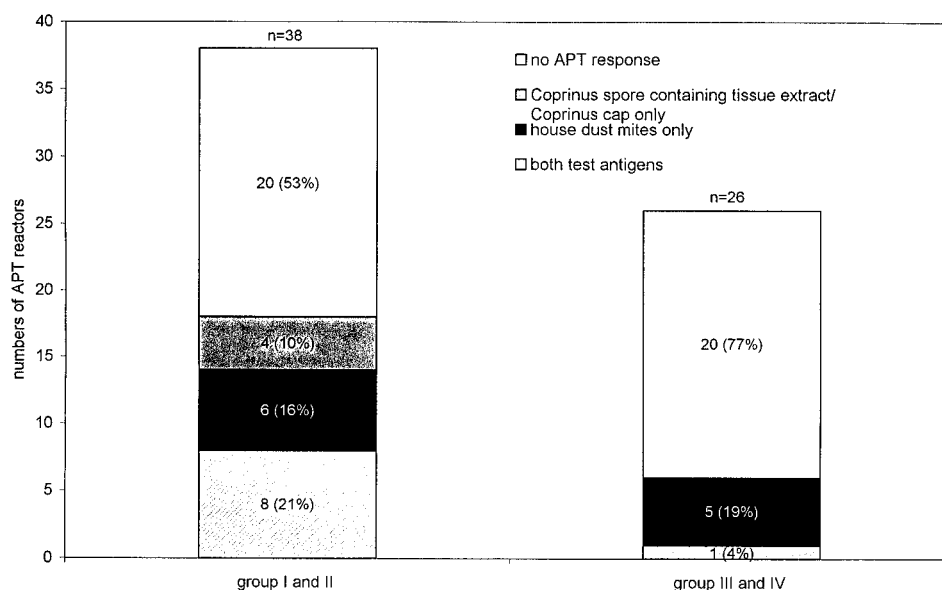


FIG 3. Comparison of APT reactivity to *C. comatus* and house dust mite allergen in subjects with AD (groups 1 and 2) and with respiratory allergy (groups 3 and 4).

to those present in spore extracts and that allergens isolated from caps share many of the same determinants with spore allergens.²⁸ Reduced activity of CSCTE may be related to the autolytic process from enzymes released during extract preparation²⁸ or to the extraction method used, resulting either in a lower allergen concentration or an extraction buffer-restricted cluster of allergens compared with the native ground cap.

In our series most APT-responsive subjects with AD showed a delayed-type hypersensitivity reaction to both the house dust mite allergen and the mushroom allergens. Only a few subjects reacted exclusively to either *C. comatus* or house dust mite allergen. The similar incidence of SPT and APT reactivity to both inhalants suggests that mushroom allergens could be as relevant as house dust mite allergens in patients with AD.

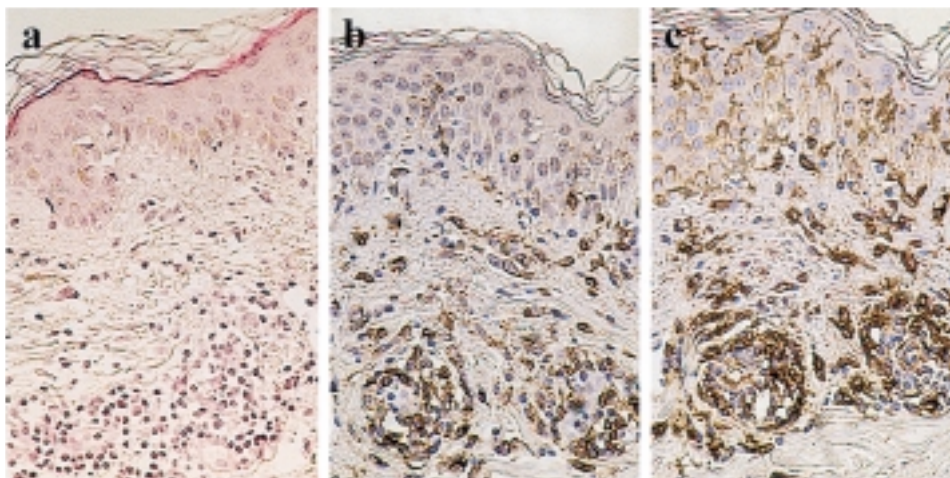


FIG 4. Histologic (**a**) and immunohistochemical (**b** and **c**) analysis of a positive APT reaction to *C comatus* cap. Focal epidermal spongiosis and a superficial, predominantly dermal mononuclear cell infiltrate is seen in **a**. Immunohistochemical staining showed a predominately CD4⁺ cell infiltrate (**b**). The majority of the mononuclear cell infiltrate was strongly stained for HLA-DR (**c**). Original magnification $\times 250$.

For decades, aeroallergens have been well-established causes of IgE-mediated respiratory allergies. Beyond that, sources of aeroallergens, such as house dust mites, animal dander, and pollen, have been shown to elicit a delayed T cell-mediated hypersensitivity skin reaction in a subgroup of individuals with AD.¹⁰⁻¹⁴ To what extent APTs detect allergens causal for AD or uncover only an enhanced skin reactivity to different allergens is still unclear. A positive APT response to an inhalant allergen does not implicate clinical relevance by itself. However, in symptomatic individuals with positive APTs, symptom reduction has been shown when the appropriate aeroallergen has been avoided.¹² Indeed, several criteria suggest that positive APT responses may point to clinical relevance. First, allergen specificity of the APT reactions has been demonstrated by cloning allergen-specific T cells from positive APT reaction sites.³⁰⁻³² Presumably an abnormal epidermal barrier function with increased transepidermal water loss allows allergens to easily penetrate the skin and to interact with antigen-presenting cells and T cells.³³ Subsequently, it is supposed that IgE on Langerhans' cells increases their antigen-presenting capacity. Second, histology of the skin lesions between APT and AD is similar, indicating that pathogenic mechanisms are comparable. Third, from a clinical point of view, individuals with an air-exposed pattern of AD are the most reactive to APTs. AD or *angry back*-like flares after APT application (unpublished observation) also endorse a relationship between APT and appearance of symptoms. Fourth, although concordance of positive SPT and APT reactivity commonly is high, aeroallergen sensitization not detected by SPT may be revealed by APT.

In summary, cap tissue of *C comatus* provides a useful source of allergens facilitating the preparations of appropriate test material for APTs and identifying subjects

sensitized to this basidiomycete. *Coprinus* species and thus other basidiomycetes may be of importance in a subgroup of patients with AD. Our findings corroborate other studies investigating other aeroallergens as triggers of AD.¹⁰⁻¹⁴

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